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Synthesis, Characterization, and Antioxidant Activity of Some Ebselen Analogues

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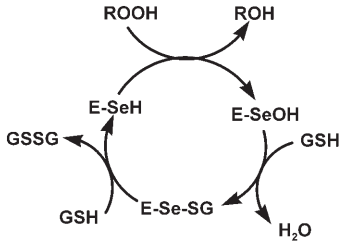
Abstract: Simple synthetic routes for several analogues of the anti-inflammatory organoselenium drug, ebselen, are described. The compounds are characterized by ^{1}H, ^{13}C, and ^{77}Se NMR spectroscopy and mass spectral techniques and, in some cases, by single-crystal X-ray diffraction studies. The glutathione peroxidase (GPx)-like antioxidant activity has been studied by using H_2O_2, *t*BuOOH, and Cum-OOH as substrates, and thiophenol (PhSH, 4-Me-C_6H_4SH) and glutathione (GSH) as cosubstrates. Density functional theory (DFT) calculations have been performed on these systems to understand the effects of various substituents on the ^{77}Se NMR chemical shifts; these results have been compared with the experimental data. The experimental and theoretical results suggest that the presence of a phenyl substituent on the nitrogen atom is important for the antioxidant activity of ebselen. While ebselen and its analogues are poor catalysts in aromatic thiol assays, these compounds exhibit high GPx activity when GSH is used as the cosubstrate. The poor catalytic activity of ebselen analogues in the presence of aromatic thiols such as PhSH and 4-Me-C_6H_4SH can be ascribed to the undesired thiol exchange reaction that takes place at the selenium center due to Se···O nonbonding interactions. To understand the effects of different peroxides on the catalytic activities, we have determined the initial rates at various concentrations of GSH and peroxides. These data suggest that the nature of peroxide has little effect on the catalytic efficiencies, although the initial reaction rates observed with hydrogen peroxide were found to be higher than that with *t*BuOOH and Cum-OOH. In contrast to the effect of peroxides, the nature of thiols appears to have a dramatic effect on the catalytic activity of ebselen and its related derivatives.

Keywords: antioxidant activity · ebselen · enzymes · selenium · selenoenzymes

Introduction

Selenium is an essential trace element that has provoked considerable interest owing to the recent identification of prokaryotic and eukaryotic enzymes containing the 21st amino acid, selenocysteine.[1] Because of the specific redox properties of selenium, the presence of a selenol group, instead of a thiol, at the active site of an enzyme confers a dramatic catalytic advantage. In mammals, selenium exerts its biological effect mainly in selenoenzymes that include glutathione peroxidase (GPx), iodothyronine deiodinase (ID), and thioredoxin reductase (TrxR). Glutathione peroxidase is an antioxidant enzyme that protects biomembranes and other cellular components from oxidative damage by catalyzing the reduction of a variety of hydroperoxides (ROOH); it uses GSH as the reducing substrate (Scheme 1).[2] Type I iodothyronine deiodinase (ID-I), a deiodinase enzyme containing selenium, catalyses the deiodination of thyroxine (T4) to activate the thyroid hormones, and links the thyroid status with selenium and iodine levels.[3] Thioredoxin reductase, on the other hand, is a dimeric flavoenzyme that catalyzes the reduction of thioredoxin (Trx)



Scheme 1. Proposed catalytic mechanism for the reduction of hydroperoxides by GPx.

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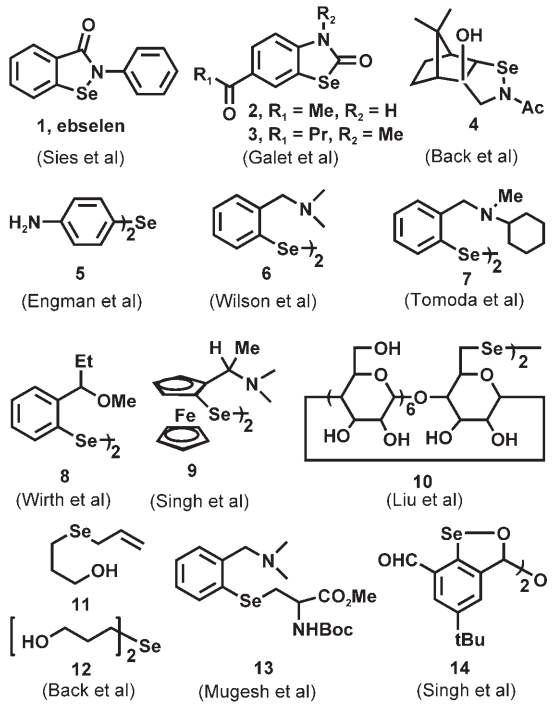
by using NADPH as the cofactor.[4] This reaction forms the basis for a number of further processes such as the synthesis of deoxyribonucleotides, defense against oxidative stress,[5] redox regulation of gene expression, and signal transduction.[6]

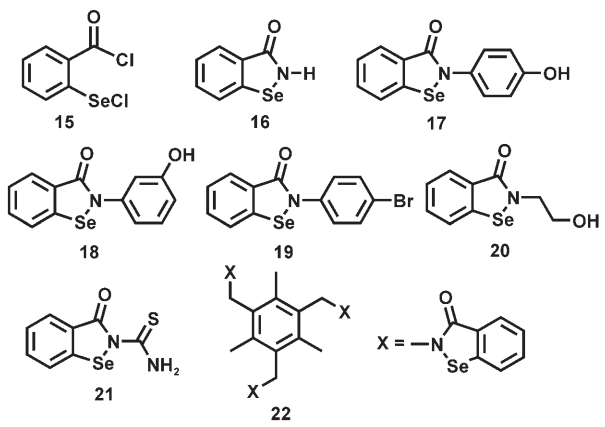
The chemistry at the active site of GPx has been extensively investigated with the help of synthetic selenium compounds. Since the discovery that ebselen (2-phenyl-1,2-benzoisoselenazol-3-(2H)-one, **1**) exhibits significant antioxidant activity by mimicking the active site of GPx,[7] several groups have pursued the design and synthesis of low-molecular-weight GPx mimics, either by modifying the basic structure of ebselen, or by incorporating some structural features of the native enzyme.[7c,d] The synthetic GPx mimics reported in the literature include benzoselenazolinones (**2**, **3**),[8] selenenamide (**4**),[9] diaryl selenide (**5**),[10] various diselenides (**6**--**10**),[11] hydroxyalkyl selenides (**11**, **12**),[12] a selenocysteine derivative (**13**)[13] and selenenate ester (**14**).[14] Although several mechanisms have been proposed to account for the GPx-like behavior of ebselen, the available data suggest that ebselen and its related compounds express their GPx activity mainly by the generation of catalytically active selenols. The formation of a reactive selenol species is also required for diselenides to exhibit antioxidant activities. Back et al. have shown that the hydroxyalkyl selenides do not produce any selenol, but that they undergo facile oxidation with organic peroxides to produce cyclic seleninates or spirodioxaselenanonane as the catalytically active species.[12] The selenocysteine derivative **13**, on the other hand, has been shown to undergo oxidation followed by elimination reactions to produce a selenenic acid, which in turn reacts with thiols to generate the corresponding selenol.[13]



Although ebselen exhibits interesting therapeutic properties, including anti-inflammatory activity,[7] it is a relatively inefficient catalyst for the in vitro reduction of hydroperoxides with aryl and benzylic thiols (such as PhSH and BnSH) as cosubstrates.[12,15] The relatively poor GPx-like antioxidant activity has been ascribed to undesired thiol exchange reactions that take place at the selenium center in the selenenyl sulfide intermediate.[15] However, ebselen has been shown to be a good antioxidant in vivo, as it exhibits significant GPx activity in the presence of natural thiols such as GSH. It has been recently shown that ebselen can act either beneficially as a peroxidase mimic, or detrimentally through the depletion of GSH.[16] In the presence of a high concentration of GSH, the effect of ebselen will be primarily beneficial, while in a system in which the concentration of GSH is very low, the harmful effects (i.e., depleting GSH) may dominate. These facts may account for the discrepancies observed in the outcomes of different studies with ebselen. It has been shown that thiol cosubstrates with chemical properties that enhance the conversion of the selenenyl sulfide intermediate to the selenol form increase its catalytic activity.[15] The in vivo protective effects of ebselen might also result from the greater variety of thiol cosubstrates available, thus enhancing the formation of the catalytically active form of ebselen. We have synthesized and evaluated a number of ebselen analogues in order to understand the effects of various substituents on the GPx activity of ebselen. In addition, we describe the effects of different peroxides on the catalytic activity of these analogues.

Results and Discussion

Selenenyl chloride **15**, synthesized from anthranilic acid and disodium diselenide (Na_2Se_2),[17] was used as a key intermediate for the synthesis of most of the compounds in the present study. The other key compound **16** was synthesized by treating **15** with an aqueous solution of ammonia. Compounds **17–20** were synthesized in good yield by treating **15**



with appropriate primary amines in dry acetonitrile. The reaction of **15** with thiourea produced the monosubstituted thiourea compound **21** in moderate yield rather than the expected bis-ebselen derivative. Similarly, our attempts to synthesize compounds having two ebselen moieties by using **15** with ethylene diamine, 1,6-diamino hexane, or *p*-phenylene diamine were unsuccessful and, in each case, only the corresponding monosubstituted compound was obtained as the major product. However, these derivatives could not be purified by column chromatography due to solubility problems. The tris-ebselen compound **22** was achieved by functionalization of the free N–H group in compound **16** with 1,3,5-tri(bromomethyl)mesitylene. All the compounds in the present study were characterized by ^{1}H, ^{13}C, and ^{77}Se NMR spectroscopy and mass spectrometry techniques and, in some cases, by single-crystal X-ray diffraction studies.

Recently, ab initio and density functional theory (DFT) methods have been used to explain the redox chemistry of small-molecule GPx mimics.[15,18] It is now well established that ^{77}Se NMR spectroscopy is a very useful technique for understanding the electronic environment around the selenium atom. The experimental and/or theoretical ^{77}Se NMR chemical shifts can be used reliably for probing the nature of selenium species in solution. Therefore, we performed DFT calculations for some of the compounds in this study to understand the effects of various substituents on the ^{77}Se NMR chemical shift, and compared these calculated values with the experimental data. The geometries were fully optimized at B3LYP level of theory by using the 6–31G(d) basis sets. The NMR calculations were performed at B3LYP/6—311+G(d,p) level on B3LYP/6–31G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method. The bond lengths and angles and the ^{77}Se NMR chemical shifts obtained by the DFT calculations were compared with the experimental values; selected bond lengths and natural bond orbital (NBO) charges on selenium along with the ^{77}Se NMR chemical shifts are summarized in Table 1. The ORTEP diagrams of the crystal structures of **16–20** and the corresponding DFT-optimized geometries are given in Figure 1.

As cleavage of the Se–N bond by thiols is important for the GPx activity of ebselen and related compounds, we compared the Se–N bond lengths of **16–20** with that of ebselen.

Table 1. Summary of DFT calculations on 1, 16–20 at the B3LYP/6–31G(d) level and GIAO ^{77}Se NMR chemical shifts calculated at the B3LYP/6–31G(d)//B3LYP/6–311+G(d,p) level along with experimental ^{77}Se NMR chemical shifts.

Compound	r_{Se-N} [Å]	q_{se} (Calcd)	^{77}Se δ [ppm][a]
1	1.893 (1.896)	0.622	941 (960)
16	1.869 (1.860)	0.616	845 (793)
17	1.891 (1.868)	0.620	946 (912)
18	1.893 (1.877)	0.626	945 (971)
19	1.894 (1.890)	0.620	950 (964)
20	1.881 (1.872)	0.634	889 (928)

[a] Referenced to the peak for Me_2Se. The experimental values are given in parentheses.

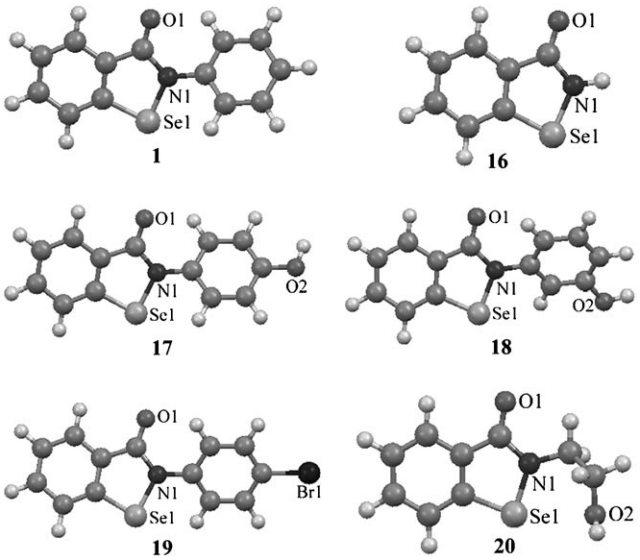


Figure 1. Optimized geometries for compounds **1** and **16–20**. Selected bond lengths and bond angles for these compounds are summarized in Table 1.

The replacement of the phenyl ring in ebselen with a hydrogen atom (**16**) appears to decrease the bond length. The shorter Se–N bond length led to a significant upfield shift in the ^{77}Se NMR spectrum of **16**: the signal observed at $\delta =$ 793 ppm with respect to Me_2Se is shifted upfield when compared with that of ebselen ($\delta = 961$ ppm). The replacement of the phenyl group of ebselen with a CH_2CH_2OH moiety (**20**) or the substituents on the phenyl ring (**17–19**) does not have any significant effect on the Se–N distances or the ^{77}Se NMR chemical shifts. In **20** a weak intramolecular Se···O nonbonding interaction (Se···O: 2.902 Å, X-ray; 2.954 Å, calcd) was observed both in the crystal structure and optimized geometry, which may have some effect on the Se–N bond length.

The GPx-like activity of ebselen and its related derivatives was studied by using hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (*t*BuOOH), and cumene hydroperoxide (Cum-OOH) as substrates, and GSH, PhSH, and 4-MeC_6H_4SH as thiol cosubstrates. The catalytic activity with GSH of the test compounds was studied in a classical GSH–GSSG coupled assay,[11a] by using UV/Vis spectrophotometry, and the activity with aromatic thiols was studied by reversed-phase HPLC. The initial rates (v_0) for the reduction of hydroperoxide by thiols in the presence and absence of test compounds were calculated from a linear fit spanning the first 5–10% of the reaction (Table 2). The catalytic rates were corrected for the background reaction between peroxides and thiols. In agreement with previous reports, ebselen was found to be a poor catalyst in the in vitro reduction of hydroperoxides by aromatic thiols.[15] Similarly, **16–20**, bearing the basic ebselen moiety, were also found to be poor catalysts in the PhSH and 4-MeC_6H_4SH assays (Table 2).

As in the case for ebselen, the relatively low catalytic activity of these compounds in the aromatic thiol assays can

Table 2. Initial rates (v_0) for the reduction of hydrogen peroxide and organic peroxide by benzenethiol (5 mM) in the presence of ebselen analogues (0.5 mM) at 18 °C.

Compound	Initial rates, v_0 [μM min^{-1}][a]		
	H_2O_2	*t*BuOOH	Cum-OOH
1	8.5 ± 0.1	16.1 ± 0.2	7.9 ± 0.3
16	6.5 ± 0.1	11.0 ± 0.4	7.0 ± 0.1
17	9.6 ± 0.7	8.6 ± 0.5	10.1 ± 0.2
18	8.8 ± 0.6	12.8 ± 0.5	10.5 ± 0.5
19	14.6 ± 0.1	6.1 ± 0.1	8.7 ± 0.3
20	10.8 ± 0.1	7.4 ± 0.3	8.6 ± 0.4

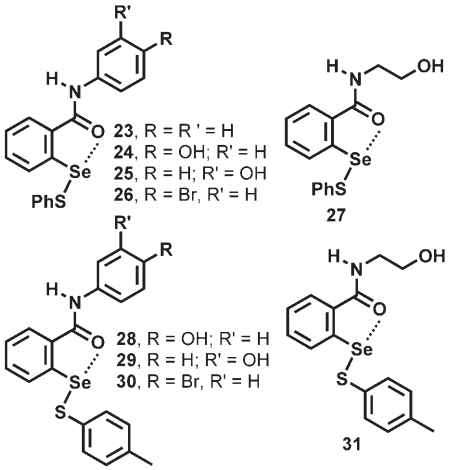
[a] Assay condition: test compound (0.5 mM), PhSH (5 mM), H_2O_2 (10 mM), *t*BuOOH (10 mM) and Cum-OOH (10 mM) in MeOH.

be attributed to the presence of strong Se···O noncovalent interactions in the selenenyl sulfide intermediates (Table 3), which prevent the regeneration of catalytically active selenol

Table 3. Summary of DFT calculations on **23–27** at the B3LYP/6–31G(d) level and NBO analysis at B3LYP/6–31G(d) level.

Compound	$r_{Se\cdots O}$ [Å]	$\theta_{S\text{-}Se\text{-}O}$ [°]	q_{Se}	$E_{Se\cdots O}$ [kcal mol^{-1}]
23	2.470	177.3	0.377	19.03
24	2.464	177.4	0.374	19.61
25	2.467	177.4	0.376	19.31
26	2.478	177.3	0.379	18.26
27	2.450	177.5	0.376	21.17

species due to an undesired thiol exchange reaction at the selenium center. The presence of such interactions was confirmed by DFT studies; these suggest that the nonbonding Se···O interactions increase the nucleophilic attack at the selenium atoms and that the strength of these interactions depends upon the substituents attached to the phenyl ring of ebselen. The selenenyl sulfide derived from ebselen (**23**) and the other selenenyl sulfides (**24–27**) showed strong Se···O interactions. To confirm the interactions in solution, we synthesized **28–31** from the corresponding cyclic compounds (**17–20**) by treatment with 4-MeC_6H_4SH. The ^{77}Se NMR of **28–31** showed a large downfield shift compared



with PhSeSPh, thus supporting the results of the DFT calculations.

In accordance with our previous report[15] and others,[7] ebselen exhibited considerable GPx activity when GSH was used as the cosubstrate. The other ebselen derivatives also exhibited significant catalytic activity with GSH. In all three peroxide systems, the catalytic activities of **17**, **18**, and **20–22** were found to be much higher than that of ebselen (Table 4). The lower catalytic activity of **16** (compared to

Table 4. Initial rates (v_0) for the reduction hydrogen peroxide and organic peroxides by glutathione (2 mM) in the presence of ebselen analogues (80 μM) at 23 °C.

Compound	Initial rates, v_0 [μM min^{-1}][a]		
	H_2O_2	*t*BuOOH	Cum-OOH
1	140.3 ± 1.6	86.1 ± 1.0	88.2 ± 0.1
16	103.0 ± 0.5	59.0 ± 2.4	87.3 ± 2.4
17	278.0 ± 1.3	169.1 ± 2.9	266.8 ± 1.7
18	257.7 ± 0.3	142.6 ± 0.7	231.8 ± 2.7
19	71.2 ± 0.8	29.8 ± 0.6	45.8 ± 2.4
20	179.1 ± 1.7	124.2 ± 1.3	143.4 ± 0.4
21	337.8 ± 0.1	216.1 ± 2.9	330.7 ± 2.4
22	253.6 ± 1.3	177.0 ± 2.5	213.9 ± 2.0

[a] Assay conditions: phosphate buffer (100 mM), glutathione reduced (2 mM), NADPH (0.4 mM), EDTA (1 mM), glutathione Reductase (1 unit), peroxide (1.6 mM) and test compound (80 μM).

ebselen) suggests that a substitution at the nitrogen is required for high GPx activity. The tris-ebselen compound **22** exhibited high GPx activity, although the initial rates were found to be only two times higher than that of ebselen. To determine the relative orientation of the ebselen moieties in the molecule, we undertook single-crystal X-ray studies (Figure 2). These showed that all three ebselen moieties are oriented perpendicular to the mesitylene ring. It appears that the relative orientation of the three ebselen units leads to steric hindrance around each selenium atom, which may reduce GPx activity.

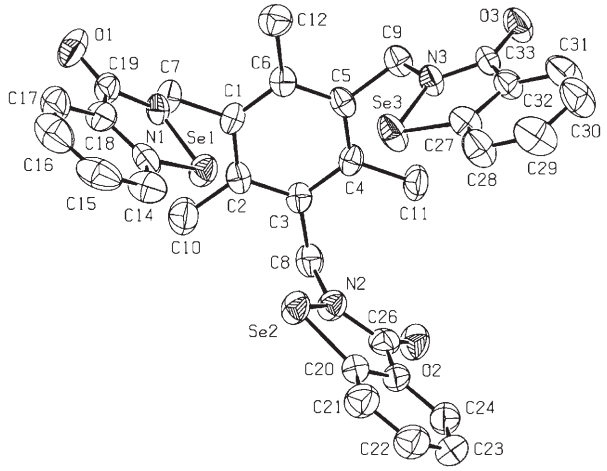


Figure 2. Single-crystal X-ray structure of **22** (including 50% probability ellipsoids). The hydrogen atoms are omitted for clarity.

While the differences in the thiol cosubstrates had a dramatic effect on the catalytic activity of ebselen and its analogues, a change in the peroxide substrate did not have a significant effect. In general, the initial rates for the reduction of H_2O_2 and Cum-OOH were found to be higher than that of *t*BuOOH (Table 4). To understand the effects of different peroxides on the catalytic activities, we determined the initial rates at various concentrations of GSH and peroxides. The Lineweaver–Burk (double-reciprocal) plots for **17**, obtained by plotting the reciprocal of initial rate ($1/v_0$) against the reciprocal of substrate concentration (1/[substrate]), were used for the determination of the catalytic parameters. The plots obtained for various concentrations of GSH at fixed concentration of H_2O_2, *t*BuOOH, and Cum-OOH are summarized in Figure 3, and the catalytic parameters determined for both peroxide and GSH variations are summarized in Table 5. Although the initial rate for the reduction of *t*BuOOH in the presence of **17** was found to be lower than that of H_2O_2 and Cum-OOH, the catalytic efficiencies (η) determined for *t*BuOOH were almost identical to those for H_2O_2 and Cum-OOH. Similarly, the catalytic efficiencies determined at various thiol concentrations for each peroxide concentration were found to be comparable to the corresponding values at equivalent concentrations of the peroxides. These observations reveal that the nature of peroxide does not affect the GPx activity of small-molecule GPx mimics.

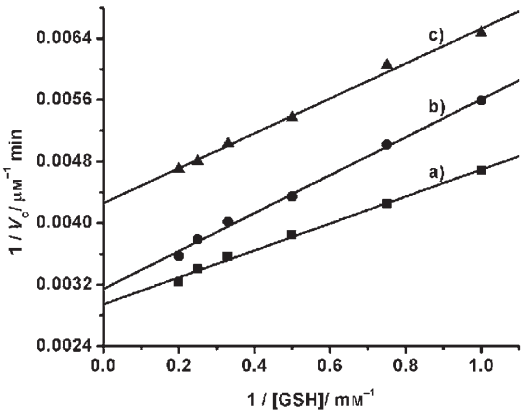


Figure 3. Lineweaver–Burk plots obtained for **17** at various concentrations of GSH, at fixed concentration of H_2O_2 (line a), Cum-OOH (line b), and *t*BuOOH (line c). The concentrations of peroxide and selenium catalyst were 1.6 mM and 80 μM, respectively.

It should be mentioned that the GPx super-family contains four types of enzymes: the classical cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx), and gastrointestinal GPx (giGPx). All require selenium at their active sites for catalytic activity. While the thiol cosubstrate specificity of these enzymes is highly specific, the hydroperoxide substrate specificity is very broad: these enzymes accept a variety of peroxide substrates, including H_2O_2 and a number of organic hydroperoxides such as *t*BuOOH and Cum-OOH. Therefore, it is not surprising that the synthetic mimics exhibited good GPx activity in all three peroxide assays. However, the identification of thiol cofactor systems with superior reducing ability is essential for an understanding the in vivo antioxidant activity of ebselen and its related compounds.

Table 5. Effect of peroxide and thiol concentrations on the maximum velocity (V_{max}), Michaelis constant (K_m), catalytic constant (K_{cat}), and catalytic efficiency (η) for catalyst **17**.

Peroxide	V_{max} [μM min^{-1}]	K_m [mM]	K_{cat} [min^{-1}]	η [M^{-1} min^{-1}]
effect of peroxide concentration[a]				
H_2O_2	387.6	0.511	4.84	9.48×10^3
*t*BuOOH	265.9	0.354	3.32	9.39×10^3
Cum-OOH	299.4	0.413	3.74	9.06×10^3
effect of thiol concentration[b]				
H_2O_2	339.0	0.590	4.24	7.18×10^3
*t*BuOOH	234.7	0.533	2.93	5.50×10^3
Cum-OOH	318.5	0.783	3.98	5.08×10^3

[a] Assay conditions: phosphate buffer (100 mM), glutathione reduced (2 mM), NADPH (0.4 mM), EDTA (1 mM), glutathione reductase (1 unit), peroxide (variable), compound **17** (80 μM). [b] Assay conditions: phosphate buffer (100 mM), glutathione reduced (variable), NADPH (0.4 mM), EDTA (1 mM), glutathione reductase (1 unit), peroxide (1.6 mM), test compound **17** (80 μM).

Conclusion

The present study of the GPx activity of ebselen and its related compounds suggests that the presence of a phenyl substituent on the nitrogen atom is important for the antioxidant activity of ebselen. This study also suggests that the strength of the Se–N covalent bond does not have a significant effect on the antioxidant activity. However, the Se···O noncovalent interactions in the selenenyl sulfide intermediates do alter the catalytic activity. Compounds that produce selenenyl sulfides with strong Se···O interactions are found to be less active compared with those with weaker Se···O interactions. Our studies of the effects of various hydroperoxides suggest that the nature of the peroxide has little effect on the catalytic efficiencies, although the initial reaction rates observed with hydrogen peroxide were found to be higher than those with *t*BuOOH and Cum-OOH. The nature of the thiols, on the other hand, showed a dramatic effect on the catalytic activity of ebselen and its analogues, thus indicating that the discrepancies observed in the outcomes of different studies were probably due to the nature of the thiol systems, and not to the differences in the peroxides employed for the assays.

Experimental Section

General procedure: All reactions were carried out in a nitrogen atmosphere by using standard vacuum-line techniques. Acetonitrile and DMF were dried over P_2O_5, and THF was dried over sodium metal with benzo-

phenone. Compound **1**, was synthesized by following a published method.[19]

^{1}H (400 MHz), ^{13}C (100.56 MHz), and ^{77}Se (76.29 MHz) NMR spectra were obtained at room temperature on a 400 MHz NMR spectrometer (Bruker Optik, Ettlingen, Germany). Chemical shifts are quoted with respect to $SiMe_4$ as internal (^{1}H and ^{13}C), and Me_2Se as external (^{77}Se) standards. A Perkin–Elmer Lambda 5 UV/Vis spectrophotometer was used to measure GPx activity. The melting points (uncorrected) of the compounds were determined in an open capillary with a B-540 Melting Point apparatus (Büchi Labortechnik AG, Flawil, Switzerland). Mass spectral studies were carried out on a Q-TOF micro mass spectrometer (Waters Inc, Milford, MA, USA) with ESI MS mode analysis. Elemental analyses were performed on a ThermoFinigan FLASH EA 1112 CHNS analyzer (Thermo Fisher Scientific Inc, Waltham, MA, USA).

Synthesis of 16: A solution of ammonia (25% in water, 0.55 mL, 6.49 mmol) was added dropwise to a stirred solution of 2-(chloroseleno)-benzoyl chloride (0.500 g, 1.96 mmol) in dry acetonitrile (20 mL) over 10 min. The mixture was then stirred at room temperature for 1 h, and the solvent was evaporated in vacuo. Water (50 mL) was added and the mixture was stirred for a further 2 h at room temperature. The precipitated solid was filtered off and purified by active neutral alumina column chromatography by using ethyl acetate and methanol (9:1). The resulting white solid was recrystallized from methanol to produce colorless needles. Yield: 0.31 g (81%); m.p: 222–224°C; ^{1}H NMR ([D$_6$]DMSO): δ = 7.46–7.50 (t, ^3J = 7.2 Hz, 1H), 7.65–7.69 (t, ^3J = 7.6 Hz, 1H), 7.87–7.88 (d, ^3J = 7.6 Hz, 1H), 8.11–8.13 (d, ^3J = 8.0 Hz, 1H), 9.27 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 126.04, 126.75, 127.69, 128.06, 131.97, 141.87, 169.16 ppm; ^{77}Se NMR ([D$_6$]DMSO): δ = 793.6 ppm; HRMS (TOF MS): m/z calcd for C_7H_5NOSe $[M+Na]^+$: 221.9434; found: 221.9440; elemental analysis calcd (%) for C_7H_5NOSe: C 42.44, H 2.54, N 7.07; found: C 42.56, H 2.99, N 7.08.

Synthesis of 17: Compound **17** was synthesized following the published method[20] with a slight modification. A solution of 2-(chloroseleno)benzoyl chloride (0.300 g, 1.18 mmol) in dry acetonitrile (10 mL) was added dropwise to a stirred solution of 4-hydroxyaniline (0.154 g, 1.41 mmol) in dry acetonitrile (7 mL) at room temperature over 10 min. The reaction mixture was then stirred at room temperature for about 2 h and the solvent was evaporated in vacuo. Water (50 mL) was added and stirring was continued for a further 12 h. The precipitate was filtered off and dried to obtain a grey solid that was purified in an active neutral alumina column, by using ethyl acetate and methanol (9:1) as eluent. The resulting grey compound was recrystallized from methanol to obtain deep red needles. Yield: 0.280 g (82%); m.p: 255–257°C; ^{1}H NMR ([D$_6$]DMSO): δ = 6.77–6.79 (d, ^3J = 6.8 Hz, 2H), 7.29–7.31 (d, ^3J = 8.4 Hz, 2H), 7.40–7.44 (t, ^3J = 7.2 Hz, 1H), 7.60–7.64 (t, ^3J = 7.6 Hz, 1H), 7.81–7.83 (d, ^3J = 7.6 Hz, 1H), 8.00–8.02 (d, ^3J = 8.0 Hz, 1H), 9.61 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 116.04, 126.22, 126.63, 127.29, 128.30, 128.73, 131.01, 132.44, 139.47, 156.22, 165.40 ppm; ^{77}Se NMR ([D$_6$]DMSO): δ = 912.0 ppm; HRMS (TOF MS): m/z calcd for $C_{13}H_9NO_2Se$ $[M+H]^+$: 291.9876; found: 291.9885; elemental analysis calcd (%) for $C_{13}H_9NO_2Se$: C 53.81, H 3.13, N 4.83; found: C 52.71, H 3.35, N 4.96.

Synthesis of 18: Compound **18** was synthesized following the published method[20c] with a slight modification. A solution of 2-(chloroseleno)benzoyl chloride (0.100 g, 0.39 mmol) in dry acetonitrile (5 mL) was added dropwise to a stirred solution of 3-hydroxyaniline (0.051 g, 0.47 mmol) in dry acetonitrile (5 mL), over 10 min. The reaction mixture was then stirred at room temperature for about 2 h and the solvent was evaporated in vacuo. Water (30 mL) was then added and stirring was continued for a further 12 h. The precipitate was filtered off and dried to obtain an off-white solid. The purification in an active neutral alumina column by using ethyl acetate and petroleum ether (2:3) as eluent gave an yellow compound which was recrystallized from methanol to obtain yellowish green needles. Yield: 0.086 g (76%); m.p: 194–196°C. ^{1}H NMR ([D$_6$]DMSO): δ = 6.57–6.59 (d, ^3J = 8 Hz, 1H), 6.95–6.97 (d, ^3J = 7.2 Hz, 1H), 7.06 (s, 1H), 7.13–7.17 (t, ^3J = 8 Hz, 1H), 7.385–7.421 (t, ^3J = 6.8 Hz, 1H), 7.58–7.62 (t, ^3J = 7.6 Hz, 1H), 7.80–7.82 (d, ^3J = 8 Hz, 1H), 7.97–7.99 (d, ^3J = 8 Hz, 1H), 9.65 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 111.85, 113.41, 115.44, 126.22, 126.74, 128.40, 129.17, 130.41, 132.72, 139.25, 141.13, 158.31, 165.29 ppm; ^{77}Se NMR ([D$_6$]DMSO): δ = 974.5 ppm; HRMS (TOF MS): m/z calcd for $C_{13}H_9NO_2Se$ $[M+Na]^+$: 313.9696; found: 313.9646; elemental analysis calcd (%)for $C_{13}H_9NO_2Se$: C 53.81, H 3.13, N 4.83; found: C 53.99, H 3.48, N 5.01.

Synthesis of 19: A solution of 2-(chloroseleno)benzoyl chloride (0.100 g, 0.39 mmol) in dry acetonitrile (5 mL) was added dropwise to a stirred solution of 4-bromoaniline (0.081 g, 0.47 mmol) in dry acetonitrile (5 mL), over 10 min, followed by addition of triethyl amine (0.10 mL, 0.78 mmol). The reaction mixture was then stirred at room temperature for about 2 h and the solvent was evaporated in vacuo. Water (30 mL) was added and stirring was continued for a further 12 h. The precipitate was filtered off and dried to obtain an off white solid. The purification in an active neutral alumina column by using ethyl acetate and petroleum ether (1:3) as eluent gave an yellow compound which was crystallized from dichloromethane and petroleum ether (1:1) to obtain colorless needles. Yield: 0.118 g (85%); m.p: 194–196°C; ^{1}H NMR ([D$_1$]$CHCl_3$): δ = 7.46–7.57 (m, 5H), 7.66 (s, 1H), 7.67 (s, 1H), 8.10–8.12 ppm (d, ^3J = 8 Hz, 1H); ^{13}C NMR ([D$_1$]$CHCl_3$): δ = 119.99, 123.82, 126.77, 126.83, 127.28, 129.50, 132.42, 132.85, 137.39, 138.26, 165.75 ppm; ^{77}Se NMR ([D$_1$]$CHCl_3$): δ = 964.8 ppm; HRMS (TOF MS): m/z calcd for $C_{13}H_8NOBrSe$ $[M+H]^+$: 353.9032; found: 353.8982; elemental analysis calcd (%) for $C_{13}H_8NOBrSe$: C 44.22, H 2.28, N 3.97; found: C 43.90, H 2.50, N 3.69.

Synthesis of 20: Compound **20** was synthesized following the published method[20] with a slight modification. A solution of 2-(chloroseleno)benzoyl chloride (0.100 g, 0.39 mmol) in dry acetonitrile (5 mL) was added dropwise to a stirred solution of 2-amino-ethanol (30 μL, 0.47 mmol) in dry acetonitrile (5 mL), over 10 min. The reaction mixture was then stirred at room temperature for about 2 h and the solvent was evaporated in vacuo and dried to obtain an off white solid. The purification in an active neutral alumina column by using petroleum ether and ethyl acetate (1:4) as eluent gave a yellow compound, which was recrystallized from methanol to obtain colorless needles. Yield: 0.073 g (77%); m.p: 152–154°C; ^{1}H NMR ([D$_4$]MeOH): δ = 3.28 (s, 1H), 3.77–3.80 (t, ^3J = 5.2 Hz, 2H), 3.91–3.94 (t, ^3J = 5.2 Hz, 2H), 7.40–7.42 (t, ^3J = 8 Hz, 1H), 7.57–7.60 (t, ^3J = 7.2 Hz, 1H), 7.88–7.92 ppm (m, 2H); ^{13}C NMR ([D$_4$]MeOH): δ = 46.56, 60.72, 124.66, 125.57, 127.19, 127.27, 131.55, 140.67, 168.25 ppm; ^{77}Se NMR ([D$_4$]MeOH): δ = 928.8 ppm; HRMS (TOF MS): m/z calcd for $C_9H_9NO_2Se$ $[M+Na]^+$: 265.9696; found: 265.4981; elemental analysis calcd (%) for $C_9H_9NO_2Se$: C 44.64, H 3.75, N 5.78; found: C 45.06, H 4.19, N 5.69.

Synthesis of 21: A solution of 2-(chloroseleno)benzoyl chloride (0.200 g, 0.79 mmol) in dry acetonitrile (10 mL) was added dropwise to a stirred solution of thiourea (0.030 g, 0.394 mmol) in dry acetonitrile (5 mL), over 10 min, followed by the addition of triethyl amine (0.20 mL, 1.57 mmol). The reaction mixture was then stirred at room temperature for about 12 h and the solvent was evaporated in vacuo. Water (30 mL) was then added and stirring was continued for a further 12 h. The precipitate was filtered off and dried to obtain a yellow solid that was purified by alumina column chromatography with methanol and ethyl acetate (1:9) to give yellow solid. Yield 0.039 g (42%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.39–7.42 (t, ^3J = 6.8 Hz, 1H), 7.64–7.68 (t, ^3J = 7.2 Hz, 1H), 7.82–7.83 (d, ^3J = 7.2 Hz, 1H), 7.92–7.94 (d, ^3J = 8 Hz, 1H), 9.93 (s, 1H), 10.15 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 125.38, 126.55, 128.98, 129.88, 134.22, 141.84, 164.93, 181.52 ppmm; ^{77}Se NMR ([D$_6$]DMSO): δ = 927.4 ppm; elemental analysis calcd (%) for $C_8H_6N_2OSSe$: C 37.36, H 2.35, N 10.89, S 12.47; found: C 37.62, H 2.90, N 10.73, S 12.41.

Synthesis of 22: An aqueous solution of KOH (90 μL, 0.80 mmol) was added dropwise to a solution of **16** (0.158 g, 0.80 mmol) in dry DMF (5 mL), and stirred for 10 min. Then a solution of 1,3,5-tribromomethyl mesitylene (0.100 g, 0.25 mmol) in dry DMF (5 mL) was added dropwise and the reaction mixture was stirred at 60°C for 36 h. The solvent was evaporated in vacuo and the white solid was purified by an active neutral alumina column by using methanol and ethyl acetate (1:4) as eluent to give a white solid which was recrystallized from a methanol and DMSO (1:1) mixture to obtain colorless crystals. Yield: 0.124 g (66%); m.p: 254–256°C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.36 (s, 9H), 4.97 (s, 6H), 7.34–7.38 (t, ^3J = 7.2 Hz, 3H), 7.50–7.53 (t, ^3J = 7.2 Hz, 3H), 7.79–7.81 (d, ^3J = 7.6 Hz, 3H), 7.97–7.99 ppm (d, ^3J = 8.0 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): δ =

16.51, 43.33, 126.33, 126.55, 127.67, 128.86, 131.77, 133.94, 139.41, 140.17, 166.46 ppm; ^{77}Se NMR ([D$_6$]DMSO): $\delta = 852.5$ ppm; HRMS (TOF MS): m/z calcd for $C_{33}H_{27}N_3O_3Se_3$ $[M+Na]^+$: 775.9446; found: 775.9450.

General procedure for the synthesis of 28–31: 4-Methylbenzenethiol (0.015 g, 0.124 mmol) was added to a stirred solution of the corresponding ebselen derivative (0.124 mmol in CH_2Cl_2). The resulting solution was stirred for 1 h at room temperature, and the solvent was evaporated under reduced pressure. The product obtained was washed with petroleum ether to remove unreacted thiol and disulfide formed during the reaction. The selenenyl sulfides were obtained as a white amorphous solid in quantitative yield.

Compound 28: M.p: 182–184 °C; ^{1}H NMR ([D$_4$]MeOH): $\delta = 2.16$ (s, 3H), 6.68–6.71 (d, $^3J = 8.8$ Hz, 2H), 6.94–6.96 (d, $^3J = 8.0$ Hz, 2H), 7.24–7.39 (m, 6H) 7.86–7.88 (d, $^3J = 8$ Hz, 1H), 8.07–8.09 ppm (d, $^3J = 8$ Hz, 1H); ^{13}C NMR ([D$_4$]MeOH): $\delta = 19.53$, 114.90, 123.27, 125.87, 127.64, 127.82, 128.38, 128.91, 129.27, 131.57, 131.73, 133.16, 136.30, 136.51, 154.66, 167.00 ppm; ^{77}Se NMR ([D$_4$]MeOH): $\delta = 588.5$ ppm; TOF MS: m/z calcd for $C_{20}H_{17}NO_2SSe$ $[M+Na]^+$: 438.0043, found: 438.0021; elemental analysis calcd (%) for $C_{20}H_{17}NO_2SSe$: C 57.97, H 4.14, N 3.38, S 7.74; found: C 57.63, H 4.52, N 3.25, S 7.62.

Compound 29: ^{1}H NMR ([D$_4$]MeOH): $\delta = 2.28$ (s, 3H), 6.59–6.61 (d, $^3J = 6.4$ Hz, 1H), 7.14–7.22 (m, 4H), 7.36–7.52 (m, 4H), 7.64–7.68 (t, $^3J = 7.2$ Hz, 1H), 8.16–8.18 (d, $^3J = 8.0$ Hz, 1H), 8.22–8.24 (d, $^3J = 7.6$ Hz, 1H), 9.56 (s, 1H), 10.49 ppm (s, 1H); ^{13}C NMR ([D$_4$]MeOH): $\delta = 22.21$, 109.74, 113.40, 128.03, 129.85, 130.62, 130.94, 131.12, 131.58, 131.78, 132.98, 133.68, 134.18, 137.59, 138.24, 139.23, 140.96, 141.42, 159.31, 167.90 ppm; ^{77}Se NMR ([D$_4$]MeOH): $\delta = 599.0$ ppm; TOF MS: m/z calcd for $C_{20}H_{17}NO_2SSe$ $[M+Na]^+$ 438.0043; found: 438.0060.

Compound 30: M.p: 150–152 °C; ^{1}H NMR ([D$_1$]CHCl$_3$): $\delta = 2.28$ (s, 3H), 7.03–7.05 (d, $^3J = 8$ Hz, 2H), 7.29–7.32 (t, $^3J = 8$ Hz, 1H), 7.39–7.41 (d, $^3J = 8$ Hz, 1H), 7.45–7.52 (m, 5H), 7.67–7.68 (d, $^3J = 4$ Hz, 1H), 7.99 (s, 1H), 8.25–8.27 ppm (d, $^3J = 8$ Hz, 1H); ^{13}C NMR ([D$_1$]CHCl$_3$): $\delta = 21.01$, 117.83, 122.11, 126.22, 126.61, 129.10, 129.58, 129.75, 131.03, 132.20, 132.54, 133.05, 136.31, 136.89, 137.88, 166.01 ppm; ^{77}Se NMR ([D$_1$]CHCl$_3$): $\delta = 601.6$ ppm; TOF MS: m/z calcd for $C_{20}H_{16}NOSBrSe$ $[M+Na]^+$: 499.9199; found: 499.9220.

Compound 31: m.p: 102–104 °C; ^{1}H NMR ([D$_4$]MeOH): $\delta = 2.34$ (s, 3H), 3.61 (s, 2H), 3.81 (s, 2H), 7.09–7.11 (d, $^3J = 8$ Hz, 2H), 7.37–7.44 (m, 3H), 7.51–7.52 (m, 2H), 7.87–7.89 (d, $^3J = 8$ Hz, 1H), 8.24–8.26 ppm (d, $^3J = 8$ Hz, 1H); ^{13}C NMR ([D$_4$]MeOH): $\delta = 19.52$, 41.60, 59.71, 125.12, 126.59, 127.11, 128.32, 128.85, 129.98, 131.03, 132.44, 135.69, 168.18 ppm; ^{77}Se NMR ([D$_4$]MeOH): $\delta = 594.0$ ppm; TOF MS: m/z calcd for $C_{16}H_{17}NO_2SeS$ $[M+Na]^+$: 390.0043, found: 390.0061; elemental analysis calcd (%) for $C_{16}H_{17}NO_2SeS$: C 52.46, H 4.68, N 3.82, S 8.75; found: C 52.32, H 4.96, N 3.85, S 8.65.

GSH–GSSG coupled assay: The GPx activity was followed spectrophotometrically. The test mixture contained thiol, EDTA (1 mM), glutathione disulfide reductase (1 unit mL^{-1}), and NADPH (0.4 mM) in 0.1 M potassium phosphate buffer (pH 7.5). GPx samples (80 μM) were added to the test mixture at room temperature and the reaction was started by the addition of peroxide (1.6 mM). The initial reduction rates were calculated from the rate of NADPH oxidation at 340 nm in a GSH assay. Each initial rate was measured at least three times and calculated from the first 5–10% of the reaction by using 6.22 mM^{-1}cm^{-1} as the molar extinction coefficient for NADPH. For the peroxidase activity, the rates were corrected for the background reaction between peroxide and thiol.

HPLC assay: We employed a mixture containing a 2:1 molar ratio of PhSH and peroxide in methanol at room temperature as our model system. Assays with and without catalyst were carried out under the same conditions. Periodically, aliquots were injected into the reverse phase column and eluted with methanol and water (90:10), and the concentrations of the product diphenyl disulfide (PhSSPh) were determined at 254 nm by using pure PhSSPh as an external standard. The amount of disulfide formed during the course of the reaction was calculated from the calibration plot for the standard (PhSSPh).

Computational methods: All calculations were performed by using the Gaussian98 suite[21] of quantum chemical programs. The hybrid Becke3–Lee–Yang–Parr (B3LYP) exchange correlation functional was applied for DFT calculations.[22] Geometries were fully optimized at the B3LYP level of theory by using the 6–31G(d) basis sets. The NMR calculations were done at B3LYP/6–311+G(d,p) level on B3LYP/6–31G(d)-level-optimized geometries by using the GIAO method.[23] Orbital interactions were analyzed by using the NBO method at B3LYP/6–31G(d) level and charges were calculated from natural population analysis (NPA).[24]

X-ray crystallography: X-ray crystallographic studies were carried out on a Bruker CCD diffractometer with graphite-monochromatized $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) controlled by a Pentium-based PC running the SMART (Version 5.05; Brucker AXS, Madison, WI, 1998) software package. Single crystals were mounted at room temperature on the ends of glass fibers, and data were collected at room temperature (291 K). The structures were solved by direct methods and refined by using the SHELXTL software package.[25] In general, all non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned at idealized locations. Empirical absorption corrections were applied to all structures by using SADABS.[26] The structure was solved by a direct method (SIR-92) and refined by a full-matrix least-squares procedure on F^2 for all reflections (SHELXL-97).[27]

Crystal data for 16: C_7H_5NOSe, $M_r = 198.08$, monoclinic, space group $P2_1/c$, $a = 14.141(2)$, $b = 12.598(17)$, $c = 12.612(17)$ Å, $\beta = 108.431(2)°$; $V = 2131(5)$ Å^3, $Z = 12$, $\rho_{calcd} = 1.852$ g cm^{-3}, GOF = 0.825, $R_1 = 0.037$, $wR_2 = 0.084$ [$I > 2\sigma(I)$]; $R_1 = 0.063$, $wR_2 = 0.099$ (all data).

Crystal data for 17: $C_{13}H_9NO_2Se$, $M_r = 290.17$, monoclinic, space group $P2_1/c$, $a = 5.5584(10)$, $b = 13.8780(26)$, $c = 14.5126(27)$ Å, $\beta = 95.876(3)°$; $V = 1113(5)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.370$ g cm^{-3}, GOF = 1.370, $R_1 = 0.045$, $wR_2 = 0.103$ [$I > 2\sigma(I)$]; $R_1 = 0.047$, $wR_2 = 0.104$ (all data).

Crystal data for 18: $C_{13}H_9NO_2Se$, $M_r = 290.17$, orthorhombic, space group $Pna2_1$, $a = 15.7085(31)$, $b = 4.6494(9)$, $c = 15.3829(30)$ Å; $V = 1123(4)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.72$ g cm^{-3}, GOF = 1.032, $R_1 = 0.031$, $wR_2 = 0.073$ [$I > 2\sigma(I)$]; $R_1 = 0.043$, $wR_2 = 0.086$ (all data).

Crystal data for 19: $C_{13}H_8NOBrSe$, $M_r = 353.1$, monoclinic, space group $P2_1/n$, $a = 4.0640(19)$, $b = 25.7348(12)$, $c = 12.3811(6)$ Å; $\beta = 99.226(5)°$; $V = 1278(15)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.83$ g cm^{-3}, GOF = 1.104, $R_1 = 0.064$, $wR_2 = 0.164$ [$I > 2\sigma(I)$]; $R_1 = 0.091$, $wR_2 = 0.177$ (all data).

Crystal data for 20: $C_9H_9NO_2Se$, $M_r = 242.11$, monoclinic, space group $P2_1/c$, $a = 7.275(13)$, $b = 8.857(16)$, $c = 14.022(26)$ Å, $\beta = 102.127(3)°$; $V = 883(6)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.852$ g cm^{-3}, GOF = 0.852, $R_1 = 0.022$, $wR_2 = 0.056$ [$I > 2\sigma(I)$]; $R_1 = 0.024$, $wR_2 = 0.057$ (all data).

Crystal data for 22: $C_{33}H_{27}N_3O_3Se_3$, $M_r = 750.50$, monoclinic, space group $P2_1/c$, $a = 8.6387(6)$, $b = 24.1949(17)$, $c = 17.4994(13)$ Å, $\beta = 119.581(0)°$; $V = 3180.86(4)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.57$ g cm^{-3}, GOF = 0.812, $R_1 = 0.040$, $wR_2 = 0.068$ [$I > 2\sigma(I)$]; $R_1 = 0.073$, $wR_2 = 0.071$ (all data).

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[1] T. C. Stadtman, *Annu. Rev. Biochem.* **1996**, *65*, 83–100.
[2] a) L. Flohe, E. A. Günzler, H. H. Schock, *FEBS Lett.* **1973**, *32*, 132–134; b) J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, W. G. Hoekstra, *Science* **1973**, *179*, 588–590.
[3] a) J. R. Arthur, F. Nicol, G. J. Beckett, *Biochem. J.* **1990**, *272*, 537–540; b) M. J. Berry, L. Banu, P. R. Larsen, *Nature*, **1991**, *349*, 438–440; c) J. Köhrle, *Methods Enzymol.* **2002**, *347*, 125–167; d) A. C. Bianco, D. Salvatore, B. Gereben, M. J. Berry, P. R. Larsen, *Endocr. Rev.* **2002**, *23*, 38–89.

[4] a) S.-R. Lee, J.-R. Kim, K.-S. Kwon, H. W. Yoon, R. L. Leveine, A. Ginsburg, S. G. Rhee, *J. Biol. Chem.* **1999**, *274*, 4722–4734; b) C. H. Williams, Jr, L. D. Arscott, S. Müller, B. W. Lennon, M. L. Ludwig, P.-F. Wang, D. M. Veine, K. Becker, R. H. Schirmer, *Eur. J. Biochem.* **2000**, *267*, 6110–6117.

[5] a) "Oxidative Stress: Introductory Remarks" H. Sies, in *Oxidative Stress* (Ed.: H. Sies) Academic Press, London, **1985**, pp. 1–8; b) H. Sies, *Angew. Chem.* **1986**, *98*, 1061–1075; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1058–1071.

[6] a) H. Nakamura, K. Nakamura, J. Yodoi, *Ann. Rev. Immunol.* **1997**, *15*, 351–369; b) E. S. Arnér, A. Holmgren, *Eur. J. Biochem.* **2000**, *267*, 6102–6109.

[7] a) A. Müller, E. Cadenas, P. Graf, H. Sies, *Biochem. Pharmacol.* **1984**, *33*, 3235–3239; b) A. Wendel, M. Fausel, H. Safayhi, G. Tiegs, R. Otter, *Biochem. Pharmacol.* **1984**, *33*, 3241–3245; c) G. Mugesh, H. B. Singh, *Chem. Soc. Rev.* **2000**, *29*, 347–357; d) G. Mugesh, W.-W. du Mont, H. Sies, *Chem. Rev.* **2001**, *101*, 2125–2179.

[8] V. Galet, J.-L. Bernier, J.-P. Hénichart, D. Lesieur, C. Abadie, L. Rochette, A. Lindenbaum, J. Chalas, J.-F. Renaud de la Faverie, B. Pfeiffer, P. Renard, *J. Med. Chem.* **1994**, *37*, 2903–2911.

[9] T. G. Back, B. P. Dyck, *J. Am. Chem. Soc.* **1997**, *119*, 2079–2083.

[10] K. Vessman, M. Ekstsröm, M. Berglund, C. M. Andersson, L. Engman, *J. Org. Chem.* **1995**, *60*, 4461–4467.

[11] a) S. R. Wilson, P. A. Zucker, R.-R. C. Huang, A. Spector, *J. Am. Chem. Soc.* **1989**, *111*, 5936–5939; b) M. Iwaoka, S. Tomoda, *J. Am. Chem. Soc.* **1994**, *116*, 2557–2561; c) T. Wirth, *Molecules*, **1998**, *3*, 164–166; d) M. L. Jauslin, T. Wirth, T. Meier, F. Schoumacher, *Hum. Mol. Genet.* **2002**, *11*, 3055–3063; e) G. Mugesh, A. Panda, H. B. Singh, N. S. Punekar, R. J. Butcher, *J. Am. Chem. Soc.* **2001**, *123*, 839–850; f) Y. Sun, Y. Mu, S. Ma, P. Gong, G. Yan, J. Liu, J. Shen, G. Luo, *Biochim. Biophys. Acta* **2005**, *1743*, 199–204.

[12] a) T. G. Back, Z. Moussa, *J. Am. Chem. Soc.* **2002**, *124*, 12104–12105; b) T. G. Back, Z. Moussa, M. Parvez, *Angew. Chem.* **2004**, *116*, 1288–1290; *Angew. Chem. Int. Ed.* **2004**, *43*, 1268–1270.

[13] P. P. Phadnis, G. Mugesh, *Org. Biomol. Chem.* **2005**, ***3***, 2476–2481.

[14] S. S. Zade, H. B. Singh, R. J. Butcher, *Angew. Chem.* **2004**, *116*, 4613–4615; *Angew. Chem. Int. Ed.* **2004**, *43*, 4513–4515.

[15] B. K. Sarma, G. Mugesh, *J. Am. Chem. Soc.* **2005**, *127*, 11477–11485.

[16] H. Shi, S. Liu, M. Miyake, K. J. Liu, *Chem. Res. Toxicol.* **2006**, *19*, 655–660, and references therein.

[17] R. Cantineau, G. Tihange, A. Plenevaux, L. Christiaens, M. Guillaume, A. Welter, N. Dereu, *J. Labelled Compd. Radiopharm.* **1986**, *23*, 59–65.

[18] a) J. K. Pearson, R. J. Boyd, *J. Phys. Chem. A.* **2006**, *110*, 8979–8985; b) S. Benkova, J. Kóňa, G. Gann, W. M. F. Fabian, *Int. J. Quantum Chem.* **2002**, *90*, 555–565; c) S. Bachrach, D. W. Demoin, M. Luk, J. V. Miller, Jr., *J. Phys. Chem.* **2004**, *108*, 4040–4046.

[19] L. Engman, A. Hallberg, *J. Org. Chem.* **1989**, *54*, 2964–2966.

[20] a) M. Osajda, J. Młochowski, *Tetrahedron*, **2002**, *58*, 7531–7537; b) A. Filipovska, G. F. Kelso, S. E. Brown, S. M. Beer, R. A. J. Smith, M. P. Murphy, *J. Biol. Chem.* **2005**, *280*, 24113–24126; c) A. Welter, H. Fischer, L. Christiaens, A. Wendel, E. Etschenberg, *Ger. Offen.* **1986**, pp. 26, Eur. Pat. Appl. CODEN: GWXXBX DE 3513070 A1 19861030 CAN 106:84181 AN 1987:84181 CAPLUS.

[21] Gaussian 98, Revision A 11.3, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**.

[22] a) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789; b) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652.

[23] W. Nakanishi, S. Hayashi, *J. Phys. Chem. A* **1999**, *103*, 6074–6081, and references therein.

[24] a) A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899–926; b) Natural Bond Orbital (NBO) Version 3.1), E. D. Glendening, J. E. Reed, J. E. Carpenter, F. Weinhold.

[25] A. Altomare, G. Cascarano, C. Giacovazzo, A. Gualardi, *J. Appl. Crystallogr.* **1993**, *26*, 343–350.

[26] a) G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467–473; b) G. M. Sheldrick, SHELX-97, Program for refinement of crystal structures, University of Göttingen (Germany), **1997**.

[27] CCDC-616461 (**16**), CCDC-616460 (**17**), CCDC-616459 (**18**), CCDC-616462 (**19**), CCDC-616463 (**20**), and CCDC-626274 (**22**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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